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THE STRUCTURE AND FUNCTION OF CLASS A BETA LACTAMASES.

Physiology

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ABSTRACT

Class A betalactamases were responsible for most of the infections in the early part of 21st century. Hence, they are the main focus in research. Our aim was to look for conserved residues in Class A betalactamases. 70 sequences were accessed from Swiss prot and subjected to multiple sequence alignment using Clustal X 2.1. Nine segments were found to be conserved. These conserved segments included the catalytic sites which were four in number. The other conserved residues may have important functions other than catalysis.

KEYWORDS

class A betalactamases, multiple sequence alignment, catalysis, conserved residues.

BACKGROUND:

Beta-lactamases are enzymes (proteins) produced by bacteria. These enzymes hydrolyze the Betalactam antibiotics used for killing the bacteria. When the Betalactam antibiotics are hydrolyzed by the betalactamases, the pathogenic bacteria thrive and cause exacerbation of the infection, which may even lead to the death of the patient.(Beta-lactamase. 2017)

Betalactamases were first identified by Abraham and Chain in 1940 even before the penicillins (the first Betalactam antibiotic) were used for clinical use. Presently, there are 850 varieties of betalactamases.(Beta-lactamase. 2017)

These enzymes/proteins can be classified either by Bush, Jacoby and Medieros classification based on substrate specificity (based on the antibiotics they hydrolyze) or by the molecular classification based on their structure which was proposed by Ambler. According to Ambler's classification, betalactamases can be classified into A, B, C, and D group of beta lactamases (Drawz, S. M., & Bonomo, R. A.2010)

A, C and D group of betalactamases are called serine proteases or serine betalactamases as they contain a serine residue which takes part in the catalysis during the hydrolysis of betalactam antibiotics. The B group are called metallo-proteases or metallo betalactamases as they have zinc molecule as part of their structure.

As betalactamases evolved, resulting in production of new betalactamases which were superior with respect to hydrolyzing novel antibiotics, while few amino acids changed in the primary segments, few segments remained conserved through the evolutionary process.

Class A beta-lactamases (Ambler classification) are the most often the topic of research and experiments compared to the other three classes. This is because, class A beta-lactamases were responsible for most of the drug resistant bacteria causing infections in the early part of twenty-first century and earlier.

The aim of our research was to look for conserved amino acid residues in class A beta-lactamases sequences downloaded from Swiss prot.

BIOINFORMATIC TOOLS AND METHODOLOGY:

I.To look for conserved residues following multiple sequence alignment using Clustal X 2.1, of sequences which are accessed from Swiss prot.

a. Retrieval of fasta sequences from Swiss prot(Reviewed sequences from Uniprot) The UniProt Knowledgebase (UniprotKB) is the centre for the collection of functional information on proteins, with accurate, consistent and rich annotation. The UniProt Knowledgebase consists of two sections: a section containing manually-annotated records with information extracted from literature and curator-evaluated computational analysis, and a section with computationally analyzed records that await full manual annotation. For the sake of continuity and name recognition, the two sections are referred to as "UniProtKB/Swiss-Prot" (reviewed, manually annotated) and "UniProtKB/TrEMBL" (unreviewed, automatically annotated), respectively.

These sequences are aligned using Clustal X 2.1.

Clustal X 2.1 application has got a general purpose multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. This application calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. One could see evolutionary relationships by viewing Cladograms or Phylograms. In other words, it provides an integrated environment for performing multiple sequence and profile alignments and analysing the results.

Another method of finding the conserved residues is by analyzing the multiple sequence alignment file using a program as described in earlier studies(Pareek, V.et al 2016) This results in a list of conserved residues gives us the information about the degree of conservation of the amino acids. If all the sequences have only one amino acid , say at the 43rd position in class A beta lactamases, then it is said to be n=1. If there are 2 amino acids at the 67th position, that is if 67 sequences have aspartate and 3 sequences have histidine, then n=2.When n=15, there are 15 different amino acids across the 70 sequences. Thus, we can observe that as the "n" value increases the degree of conservation of amino acids decreases.

Correlating the above two methods, n=1&2 are completely conserved, n=3,4,5 are fully conserved.

RESULTS: A total number of 132 reviewed sequences can be accessed in Swiss prot(Uniprot) for the enzyme beta-lactamase with E.C number 3.5.2.6.(*The Enzyme Commission number (EC number) is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze. As a system of enzyme nomenclature, every EC number is associated with a recommended name for the respective enzyme* (Enzyme Commission number. (2017))

There are 82 reviewed sequences in class A. Searching conserved residues in group A resulted in nine segments of conserved residues including the four catalytic sites. 70 sequences were selected from the 82 reviewed sequences by removing the outliers in terms of length of sequences). The conserved residues are as follows.

1. Alanine$^{40}_{(3)}$-Arginine$^{41}_{(7)}$-Valine$^{42}_{(5)}$ -glycine$^{43}_{(1)}$-Tyrosine$^{44}_{(6)}$
2. Serine$^{68}_{(1)}$-Threonine$^{69}_{(4)}$-Phenylalanine$^{70}_{(7)}$-Lysine$^{71}_{(1)}$
3. Phenylalanine$^{64}_{(2)-}$ -Proline$^{65}_{(4)}$ -Methionine$^{66}_{(6)}$
4. Cysteine$^{75}_{(4)}$-Glycine$^{76}_{(5)}$-Alanine$^{77}_{(9)}$-Valine$^{78}_{(4)}$-Leucine$^{79}_{(2)}$
5. Serine$^{128}_{(1)}$-Aspartate$^{129}_{(1)}$-Asparagine$^{130}_{(3)}$
6. Alanine$^{132}_{(2)}$-Alanine$^{133}_{(9)}$-Asparagine$^{134}_{(2)}$ -Leucine$^{135}_{(6)}$-leucine136$_{(4)-}$leucine136$_{(4)}$
7. Isoleucine$^{140}_{(4)}$-Glycine$^{141}_{(4)-}$Glycine $^{142}_{(2)}$
8. Glutamic acid$^{164}_{(1)}$-Proline$^{165}_{(9)}$-glutamic acid$^{166}_{(9)-}$Leucine167$_{(1)}$
9. Aspartic acid$^{231}_{(2)}$-Lysine $^{232}_{(2)}$-Serine$^{233}_{(2)}$-Glycine $^{234}_{(1)}$

While the superscript next to the amino acid tells us about the position of the amino acid in the sequence, the subscript tells us about the degree of conservation. For example, in all the seventy sequences, in the 68^{th} position, there is only one amino acid serine.

DISCUSSION:

There are four catalytic sites in each of the class A beta-lactamase(Majiuddin et al). When the conserved residues were listed out, it was found that there were nine segments of conserved residues. As the segments that are conserved are more than the catalytic residues, it is probable that each of them have some function other than catalysis. Majiuddin et al reports in his review that some of these could have a function. For example, few of them had a role in maintaining the conformation of the beta-lactamase, few others had a role in antibiotic specificity and contributed to drug resistance by binding with certain antibiotics so that they are hydrolyzed by the beta-lactamases. Few others (n=1) were linked to the catalytic site by means of residues which had a lower degree of conservation. It is possible that those residues with lower degree of conservation too are important from a structural point of view as they may contribute towards providing stability to the structure .(Majiuddin et al 2002)

CONCLUSION: The conserved residues have functions other than catalysis. Hence, the large number of conserved residues. Future research in the direction towards finding residues that play a role in substrate specificity and noting their degree of conservation would be an active step towards explaining the drug resistance of beta-lactamases.

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